EXHIBIT

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Research Article

Influences of Sodium Carbonate on Physicochemical Properties of Lansoprazole in Designed Multiple Coating Pellets

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Abstract. Lansoprazole (LSP), a proton-pump inhibitor, belongs to class II drug. It is especially instable to heat, light, and acidic media, indicating that fabrication of a formulation stabilizing the drug is difficult. The addition of alkaline stabilizer is the most powerful method to protect the drug in solid formulations under detrimental environment. The purpose of the study was to characterize the designed multiple coating pellets of LSP containing an alkaline stabilizer (sodium carbonate) and assess the effect of the stabilizer on the physicochemical properties of the drug. The coated pellets were prepared by layer–layer film coating with a fluid-bed coater. *In vitro* release and acid-resistance studies were carried out in simulated gastric fluid and simulated intestinal fluid, respectively. Furthermore, the moisture-uptake test was performed to evaluate the influence of sodium carbonate on the drug stability. The results indicate that the drug exists in the amorphous state or small (nanometer size) particles without crystallization even after storage at 40°C/75% for 5 months. The addition of sodium carbonate to the pellet protects the drug from degradation in simulated gastric fluid in a dose-dependent manner. The moisture absorbed into the pellets has a detrimental effect on the drug stability. The extent of drug degradation is directly correlated with the content of moisture absorption. In conclusion, these results suggest that the presence of sodium carbonate influence the physicochemical properties of LSP, and the designed multiple coating pellets enhance the drug stability.

KEY WORDS: film coating; lansoprazole; pellets; physicochemical properties; sodium carbonate.

INTRODUCTION

Proton-pump inhibitors (PPIs) have emerged as the efficacious management of choice for acid-related disorders, including gastric and duodenal ulcers and gastroesophageal reflux disease and the treatment or prevention of gastroduodenal lesions induced by nonsteroidal anti-inflammatory drugs (1,2). Chemically, the basic structure of this class of compounds consists of substituted benzimidazole ring and a substituted pyridine ring connected to each other by a methylsulfinyl chain (3). The mechanism of action of PPIs is associated with the weakly basic nature of the compounds ($pKa \approx 4$) (3). At neutral pH, PPIs exist as lipophilic prodrugs without intrinsic activity, which can cross cell membranes. When the pH is less than 4, the pyridyl nitrogen is protonated, resulting in a chemical rearrangement to form a reactive cyclic sulfonamide, the pharmacologically active form of the drug (4).

The PPIs are either imidazopyridine derivatives or substituted pyridylmethylsulfinyl benzimidazole such as omeprazole, lansoprazole (LSP), pantoprazole, rabeprazole, esomeprazole, and tenatoprazole, *etc.* An important physicochemical characteristic of PPIs is the instability to heat, light, and acidic media due to their structural features (5,6). LSP (Fig. 1), a lipophilic weak base with p*Ka* 4 (3), seems to be especially sensitive to such attack compared to the other members of PPIs (5). It is a white powder with poor water solubility and degrades rapidly in aqueous solutions at low pH values (7). Therefore, LSP needs to be protected from the destructive effects of gastric acid when administered orally. To overcome the stability problems, different formulation strategies have been developed to protect this drug.

Formulation of a stable delivery system for LSP is extremely difficult. LSP belongs to class II drug, which is characterized by low solubility and high permeability. Moreover, it degrades rapidly in acidic conditions and is stable in basic environment. In general, alkaline stabilizers are added to the formulations to produce a microenvironmental pH of no less than 7. These alkalizers include sodium, potassium, calcium, magnesium, and aluminum salts of phosphoric acid and carbonic acid, citric acid, or other suitable weak inorganic or organic acid carbonate (8). However, the alkaline stabilizers used in the formulations can affect the physicochemical properties of the drug, and the related mechanisms are poorly known.

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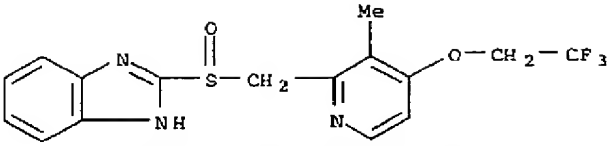

Fig. 1. The chemical structure of LSP

In addition, the solid-state form of LSP has a profound impact on solubility, stability, and bioavailability. Therefore, it should be an important consideration to design an appropriate dosage form and evaluate the physicochemical properties of LSP in formulations. In the present work, we provided an understanding of how the alkaline stabilizer affected the physicochemical properties of LSP and improved the drug stability in the novel formulation.

The major objective of the present study was to characterize the effect of sodium carbonate on the physicochemical properties of LSP in the designed pellets with multiple coating. The drug stability, acid resistance, profiles of drug release, and moisture uptake were evaluated. Differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM) were carried out for this purpose.

MATERIALS AND METHODS

Materials

LSP used was purchased from ZhuhaiRuntong Pharma Ltd. (Zhuhai, China). Non-pareil pellets (sugar spheres 0.5–0.7 mm in diameter) were provided by Gaocheng Co., Ltd. (Hangzhou, China). Hydroxypropyl methylcellulose (HPMC) 60RT5 was purchased from Feichengruitai Ltd. (Shangdong, China). Methacrylic acid copolymer used in the form of aqueous dispersion (Eudragit L30D-55) was a gift from Evonik Degussa Co., Ltd. (Darmstadt, Germany). Sodium carbonate was purchased from ErkangPharma Ltd. (Hunan, China). Triethyl citrate and talc were from Sinopharm (Shanghai, China).

Preparation of Multiple Coating Pellets of LSP

The drug-layered pellet cores were prepared by coating a layer of LSP on inert pellet cores in fluid-bed coater to achieve 25% (*w/w*) drug content. The layer of the active LSP combined with 10% (*w/w*, based on LSP) of sodium carbonate surrounds an inert core. The coating formulations were prepared by dissolving the drug into HPMC (2% *w/w*) aqueous dispersion with sodium carbonate. The dispersion was homogenized and then passed through a 60-mesh screen before coating process.

The drug-layered core pellets were coated with three successive layers, *viz.* an inner alkaline layer, a protective layer, and an enteric-coating layer, respectively (Fig. 2). Table I shows the coating formulations of alkaline, protective, and enteric layer. The coating formulations of alkaline layer were fabricated by dispersing the stabilizer into 4% (*w/w*) aqueous dispersion of HPMC. The dispersion was homogenized and then passed through a 60-mesh screen before the coating process. The preparation of protective and enteric-coating formulations was similar to the one described by Bruce *et al.* (9).

A fluid-bed coating apparatus with a Wurster container (STREA-1™ Classic, Niro-Aeromatic, Bubendorf, Switzerland) was used. Coating was performed at a batch size of 40 g, an atomizing pressure of 1.5–2.0 bar, an inlet air temperature of 45–50°C, an inlet air of 45–50 m^3/h, an exhaust air temperature of 30–35°C, a pellet bed temperature of 40–45°C, a spray rate of 1.5–2.0 mL/min, and a drying temperature of 40°C for 15 min. Finally, the pellets were dried for a further 24 h at 40°C in oven.

Acid Resistance and *In Vitro* Drug Release

To evaluate enteric protection, an acid-resistance study was performed in 500 mL of 0.1 M HCl (simulated gastric fluid, SGF, pH 1.2) for 1 h. Then, 400 mL of monobasic sodium phosphate was added to the dissolution media, and the pH was adjusted to 6.8 (simulated intestinal fluid, SIF). The drug release profiles from the coated pellets were performed with a US Pharmacopeia (USP) II apparatus (Auto SR8-Plus, Hanson research, California, USA) at 75 rpm and a temperature of 37±0.5°C. The tests were conducted in triplicate. The drug release at the acid stage was determined with a UV spectrophotometer (UV2200, Shimazu, Japan) at a wavelength of 245 nm. The drug release at buffer stage was analyzed using an HPLC (Agilent 1100 series, California, USA) assay following USP monograph procedures.

Moisture-Uptake Studies

Moisture-uptake tests of the multiple coated pellets were determined gravimetrically in triplicate. The pellets were stored in closed desiccators containing saturated solutions of KNO_3 at a relative humidity (RH) of 92.5%. At predetermined time intervals, the samples were withdrawn and accurately weighed, and the moisture uptake (%) was plotted *versus time*. The moisture absorption was calculated with the following equation:

$$\text{Moisture uptake}(\%) = \frac{W_t - W_o}{W_o} \times 100\% \qquad (1)$$

The moisture uptake (%) is the degree of moisture absorption of pellets. W_t is the weight of pellets at time t, and W_o represents the initial weight of dried pellets (before storage).

Differential Scanning Calorimetry

The thermal characteristics of the multiple coated pellets, physical mixture, and pure drug were determined by a differential scanning calorimeter (Diamond, Perkin-Elmer, USA) combined with an intercooler and nitrogen purge. Ten milligrams of pellets was weighed in aluminum pans and closed. After the usual indium and lead standard calibration, the samples were heated from 30°C to 200°C with a heating rate of 10°C/min.

Thermogravimetric Analysis

Thermogravimetric analysis of multiple coated pellets was performed with a 7–10-mg sample in aluminum pans under a dynamic nitrogen atmosphere by a thermogravimetric analyzer (Pyris6 TGA, Perkin-Elmer, USA). The experiments were run at a heating rate of 10°C/min.

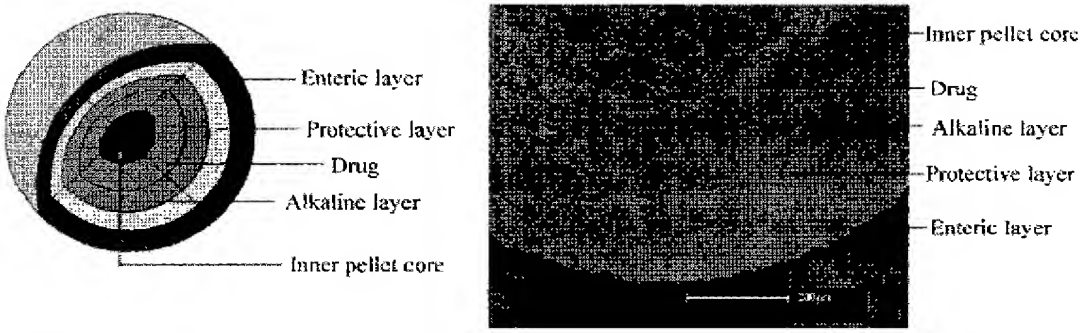

Fig. 2. The schematics of an LSP pellet with multiple coating and SEM photograph of the cross section of the pellet

Scanning Electron Microscopy

The micrographs of the samples were taken with an SEM (Hitachi S-520, Tokyo, Japan) to examine the surfaces and morphology of the multiple coating pellets, single drug-layered pellets, and the pure drug. The coated pellets were mechanically cleaved transversely and sputtered with gold for 5 min by a sputter.

Stability Studies

To assess the drug stability, the coated pellets were filled into hard gelation capsules, size 2 for analysis. The capsules were packaged with aluminum foil (Kaidi Co., Ltd., Henan, China) and stored at 40°C/75% RH for 1 and 5 months. After accelerated test, the changes in acid resistance, drug content, and drug release characteristics of the three formulations were observed. Stability samples were also analyzed by DSC and TGA.

Statistical Analysis

All the experiments were performed in triplicate. The results were expressed as mean±standard deviation. One-way analysis of variance was performed to assess the significance of the differences among the data. P values of <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Acid Resistance

LSP degrades and changes color rapidly in gastric media. Thus, enteric film coating must be developed to protect the drug from acidic conditions of gastric media. Methacrylic acid copolymer was selected as enteric film coating material since it has good moisture-protective properties, excellent enteric-coating protection in acidic media, and rapid drug release in enteric media (10). Figure 3 shows the drug release in SGF (acid resistance). As expected, the presence of sodium carbonate in alkaline layer improved the acid resistance, which is evident in the marked decrease of the drug release. Protective effect of sodium carbonate on the drug was achieved in SGF, especially evidenced by F3 since only 1% LSP was released after 60 min. Furthermore, the drug release from the pellets in SGF is reduced further as the amount of sodium carbonate is increased. The gastric stability is significantly dependent on the amount of sodium carbonate. In terms of storage stability, the pellets were stored at 40°C/75% RH for 1 and 5 months; no difference in drug release is observed ($p>0.05$) before and after storage.

In Vitro Drug Release

As an enteric formulation, the drug should be released rapidly in the intestine. Rapid drug release from the pellets enhances the bioavailability since LSP falls under class II (low solubility–high permeability) drug (11). Additionally, according to the specification for an enteric formulation from USP, more than 75% LSP should be released in SIF within 60 min. Figure 4 illustrates the drug release profiles of the pellets in SIF. Clearly, the addition of sodium carbonate to alkaline layer decreases the drug release when compared with the pellets without sodium carbonate (F0) in alkaline layer in the first 20 min. This can be attributed to the increased hydrophobicity of film coatings and coating levels for the additional sodium carbonate. However, the drug release from pellets with sodium carbonate (F1–F3) still fulfills the requirements

Table I. Film Coating Formulations of Alkaline, Protective, and Enteric Layer (All Quantities Are Given in Grams)

	Alkaline layer		Protective layer			Enteric layer		
Formulation (F)	HPMC	Na_2CO_3	HPMC	Talc	TEC	Eudragit L30D-55	Talc	TEC
F0 (control formulation)	20	–	20	6	6	40	3.6	3.6
F1	20	16	20	6	6	40	3.6	3.6
F2	20	20	20	6	6	40	3.6	3.6
F3	20	25	20	6	6	40	3.6	3.6

TEC triethyl citrate, *HPMC* hydroxypropyl methylcellulose

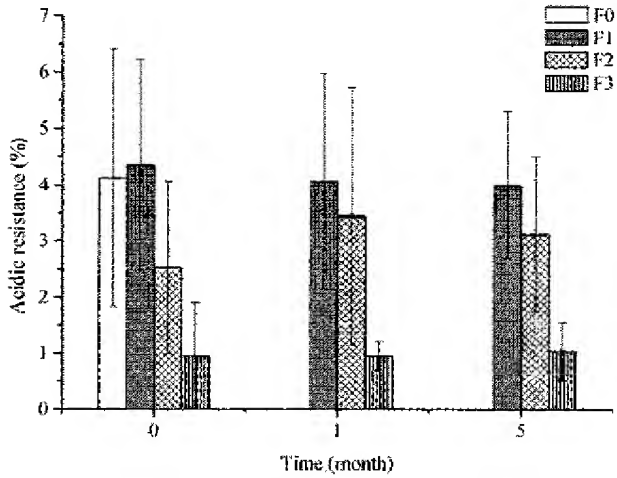

Fig. 3. Effect of amount of sodium carbonate on the acid resistance (drug release in simulated gastric fluid, pH 1.2) from multiple coating pellets before and after storage at 40°C/75% RH for 1 and 5 months (n=3). *F0*: pellets without sodium carbonate in alkaline layer. *F1*, *F2*, *F3*: pellets containing 16, 20, and 25 g sodium carbonate in alkaline layer, respectively

since more than 80% LSP is released in STF at 40 min. This can be explained by the presence of sodium carbonate: the dissolution medium permeating into the pellets dissolves the sodium carbonate, increases the microenvironmental pH, and then makes the enteric polymer ionized. This is consistent with a previous report indicating that increased pH of the subcoat facilitated the dissolution of enteric film (methyl methacrylate methacrylic acid copolymer) (12). On the other hand, the drug release is inversely proportional to the amount of sodium carbonate despite the fact that sodium carbonate has a good water solubility. The pellets with 16 g sodium carbonate (F1) shows a faster drug release (p<0.05) as compared to the pellets containing 20 and 25 g sodium carbonate (F2, F3), attributing to the least amount of sodium carbonate in F1. Interestingly, when the pellets with sodium carbonate were stored at 40°C/75% RH for 5 months, the drug release is increased. It seems that our result is not in agreement with previous investigation, indicating that a high-humidity condition resulted in decreased drug release for the lowered T_g and increased flexibility of the film (13). It is explained by the fact that sodium carbonate migrates to the enteric film and makes part of the pH-sensitive polymer ionized, causing a stretching of the polymer chain.

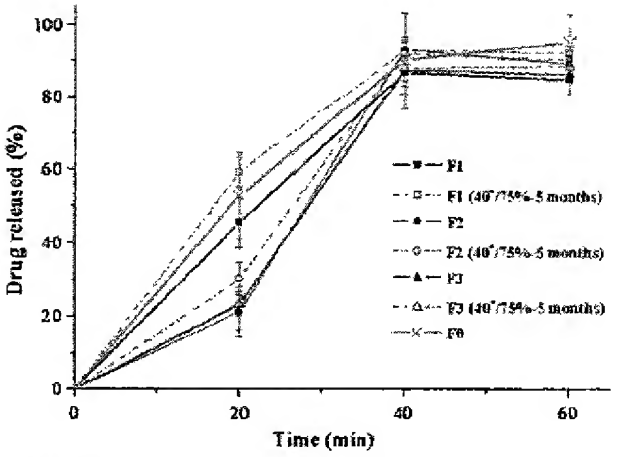

Fig. 4. Effect of amount of sodium carbonate on the drug release from multiple coating pellets before and after being stored at 40°C/75% RH for 5 months (n=3). *Solid line*: before accelerated test, *dash dot*: after accelerated test. *F0*: pellets without sodium carbonate in alkaline layer. *F1*, *F2*, *F3*: pellets containing 16, 20, and 25 g sodium carbonate in alkaline layer, respectively

Moisture-Uptake Studies

Stroyer and coworkers (8) reported that the moisture absorption plays an important role in the PPI's (omeprazole) stability when the drug is blended with enteric polymers. Thus, the moisture-uptake test was conducted in the present study. It is found that the moisture absorption increases with the following series: F0 > F1 > F2 > F3. As indicated in Fig. 5, the moisture absorption from the pellets without sodium carbonate (F0) is more than that of the pellets with sodium carbonate in alkaline layer, suggesting that the presence of sodium carbonate reduces the moisture absorption significantly (p<0.05). It is explained by the fact that the presence of sodium carbonate increases the hydrophobicity of the alkaline layer. It should be noted that the discoloration of pellets without sodium carbonate was observed after 3 days of storage. It is since that the drug particles without being surrounded by sodium carbonate migrates into the enteric layer and reacts with its acidic carboxyl groups of the polymer. In contrast, no discoloration was observed from the pellets containing sodium carbonate in alkaline layer even after 10 days of storage. Thus, the addition of sodium carbonate reduces moisture absorption and enhances the drug stability.

To confirm the moisture-uptake results, TGA was utilized to measure the moisture absorption in the pellets. Bley and coworkers (14) reported that most of the water in dosage forms will be removed at about 150°C. At this temperature, even more tightly bound water can be removed

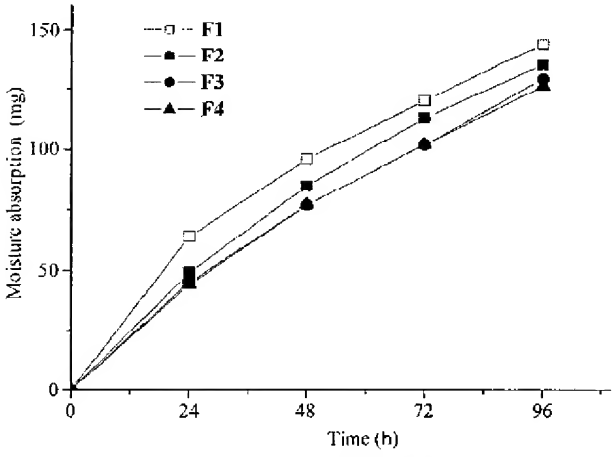

Fig. 5. Effect of amount of sodium carbonate on the moisture uptake from multiple coating pellets during open storage at 92.5% RH (n=3). *F0*: pellets without sodium carbonate in alkaline layer. *F1*, *F2*, *F3*: pellets containing 16, 20, and 25 g sodium carbonate in alkaline layer, respectively

and leveled off, resulting in a mass loss. Figure 6 shows the TGA thermograms of pellets before (Fig. 6a) and after (Fig. 6b) storage for 5 months at 40°/75% RH. With an increase of the amount of sodium carbonate, the mass loss of the pellets decreases. The mass loss increases with the following series: F1 > F2 > F3, which consists of the moisture-uptake results. Furthermore, a correlation between the moisture absorption and drug content was examined. As expected, the drug content is inversely correlated with the moisture absorption (Fig. 7). Once again, increasing the amount of sodium carbonate decreases the moisture absorption for the increased hydrophobicity of the polymer film, which contributes to the improvement of drug stability.

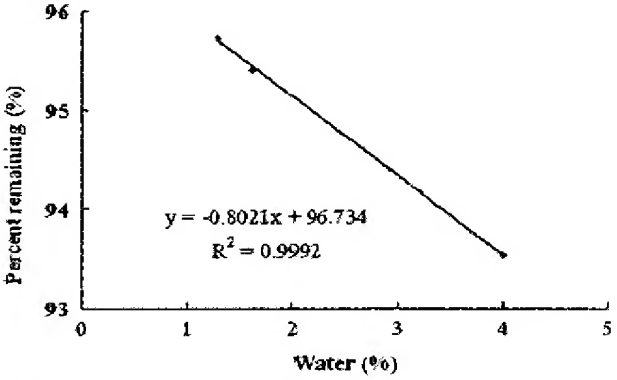

Fig. 7. Correlation between remaining drug (%) and moisture uptake (%) in multiple coating pellets after storage at 40°C/75% RH for 5 months. *F1*, *F2*, *F3*: pellets containing 16, 20, and 25 g sodium carbonate in alkaline layer, respectively

Differential Scanning Calorimetry

DSC is used to study the physical state of the drug before and after storage at 40°C/75% RH. As shown in Fig. 8a (e), the endothermic and exothermic peaks of the drug are observed (15). The physical mixtures of the drug and excipients also show the endothermic peak of LSP (Fig. 8a (d)). LSP is disordered as amorphous state and small (nanometer size) particles in the formulations, as evidenced by the absence of the melting peak in the pellets and detectable melting peak of the drug in physical mixtures.

Considering the inherent instability of amorphous solids with respect to crystallization, further thermal characteristics of the formulations were examined after storage for 5 months at 40°/75% RH. It is of interest that the melting peak is still not observed in the DSC curves (Fig. 8b), suggesting that the samples remain amorphous and small particles. This is due to the formation of

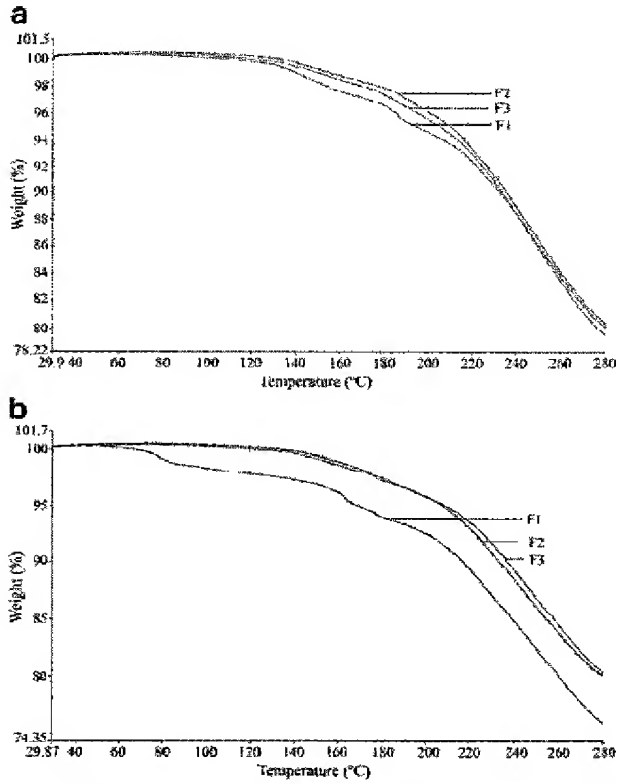

Fig. 6. DSC thermograms of multiple coating pellets containing different amount of sodium carbonate before (a) and after (b) storage at 40°C/75% RH for 5 months. (*F1*/a; *F2*/b; *F3*/c; physical mixture of LSP and the excipients/d; pure drug LSP/e). *F1*, *F2*, *F3*: pellets containing 16, 20, and 25 g sodium carbonate in alkaline layer, respectively

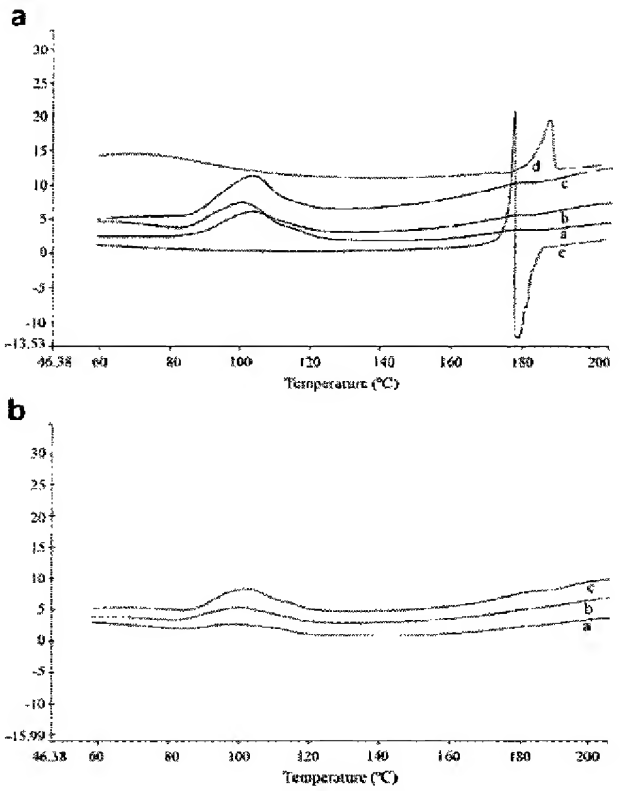

Fig. 8. TGA thermograms of multiple coating pellets containing different amount of sodium carbonate before (a) and after (b) storage at 40°C/75% RH for 5 months. *F1*, *F2*, *F3*: pellets containing 16, 20, and 25 g sodium carbonate in alkaline layer, respectively

specific drug–polymer interactions such as hydrogen bonds, which inhibit the crystallization of amorphous LSP (15,16).

Figure 9 illustrates the SEM surfaces and morphology of the single drug-layered pellet and pure drug. It is also evident that the drug is disordered as amorphous state or small particles in the pellets, since no drug crystal is observed on the surface of the pellet when compared with the pure drug.

Stability Studies

The remaining drug (%) *vs.* time relative to the initial assay is shown in Fig. 10. For the control formulation, the remaining drug is less than 80% after 1 month of storage at 40°/75% RH, and the drug could not be detected after 5 months since no basic conditions was produced. Once again, it is indicated that the stability improvement is attributed to the presence of sodium carbonate and its increased amount in alkaline layer. The addition of sodium carbonate to alkaline layer protects the drug from degradation since it creates a basic microenvironment able to stabilize the drug. Consistent with the previous results, the remaining drug (%) in formulations is in direct ratio with the amount of sodium carbonate. The resultant stability is consistent with a previous report (8), suggesting that the amount of stabilizing agent should be sufficient to capture the protons. It is also observed that the pellets containing 20 and 25 g sodium carbonate (F2 and F3) have similar stability results after 5 months of storage. This is in line with a previous report, indicating that for a weak basic compound, an increase in the pH modifier content above its minimum concentration will not achieve further drug stability (17).

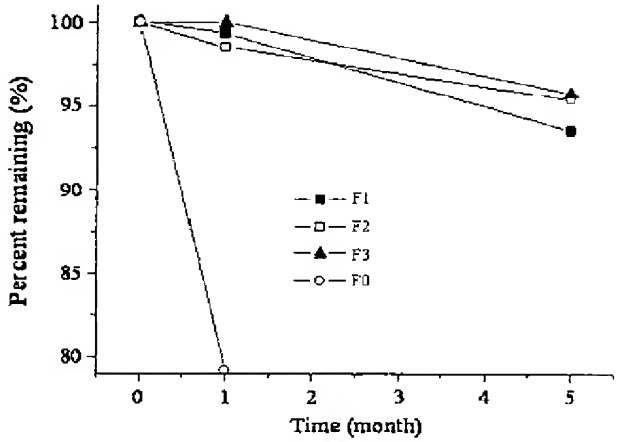

Fig. 10. Effect of amount of sodium carbonate on remaining drug (%) in multiple coating pellets after being stored at 40°C/75% RH for 1 and 5 months (n=3). *F0*: pellets without sodium carbonate in alkaline layer. *F1*, *F2*, *F3*: pellets containing 16, 20, and 25 g sodium carbonate in alkaline layer, respectively

CONCLUSIONS

The designed multiple coating pellets appear to be a promising way for stabilizing LSP. The presence of sodium carbonate in alkaline layer provides protection of the drug against degradation, and the increasing amount of sodium carbonate has a beneficial effect on the drug stability. The addition of sodium carbonate improves the gastric stability significantly. Taken together, the extent of LSP degradation is

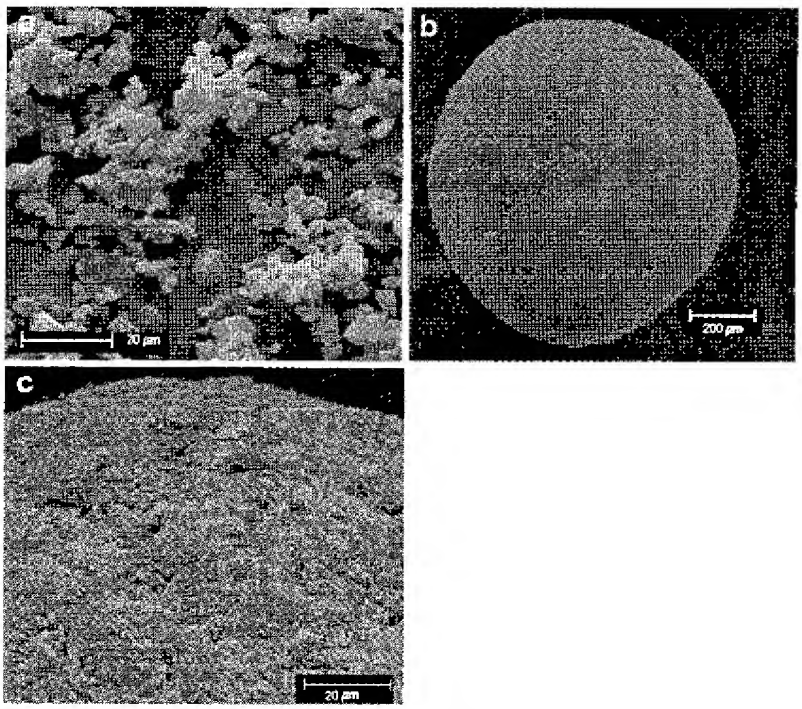

Fig. 9. SEM pictures of the surface morphology of pure LSP (a) and drug-layered pellets (b, c)

directly correlated with the moisture absorption and inversely correlated with the amount of alkaline stabilizer.

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